FACTORS INFLUENCING MOLTING AND THE SEXUAL CYCLES IN THE CRAYFISH[1]

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Introduction

There are two interesting phenomena associated with the periodic molting and the cycles of sexual functioning in crayfishes that have not been studied in detail. One of these interesting events is the delay in the spring molt of egg-carrying females until after the eggs hatch and the young crayfish leave the female; the other concerns the changes in the secondary sex characters in the male crayfish at the time of molt (Scudamore, 1942b). This investigation describes some of the factors influencing these two phenomena in the life cycle of the crayfish.

The delay in the spring molt of egg-carrying females has been observed by Van Deventer (1937), Tack (1941) and others. According to Tack (1941) the spring molt of males and non-reproducing females of the crayfish, *Cambarus immunis,* occurs about the middle of April in south central New York with most of the crayfish molting within the period of a few weeks. However, the females, which are carrying eggs at this time, do not molt until five or six weeks later. The reproducing females deposit their eggs in the fall shortly after mating and carry their eggs, attached to their abdominal pleopods, all winter. The eggs hatch about mid-May and the young remain dependent upon the female for a week or longer, while undergoing their first two molts. The egg-bearing female molts a few days after the young leave her pleopods.

The spring-molting period of males and non-reproducing females of the crayfish, *Cambarus propinquus,* also begins about the middle of April in central Illinois and lasts about three weeks (Van Deventer, 1937). However, the reproducing females do not deposit their eggs until early April. The eggs hatch about the middle of May and the young remain dependent for approximately another week. The egg-bearing females do not molt until late May or early June which is at least three weeks after the male spring-molting period.

This delay in molt of egg-bearing females is a protective adaptation, because molting earlier would result in the death of all the embryos. The mechanism producing this lag in the spring molt of ovigerous females has not been explained adequately. However, Hess (1941) reported a delay in molting of the seeded female shrimp, *Crangon armillatus,* as compared to non-seeded females. He also observed that removal of the embryos from seeded females shortened the period between molts and concluded that the factor, which inhibited molting, was apparently dependent upon attachment of the embryos to the female.

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The male secondary sex characters studied were the first pair of abdominal pleopods which are modified as gonopods for the transfer of spermatozoa to the annulus ventralis of the female during copulation. These gonopods have been described sufficiently by Turner (1926), Van Deventer (1937), Tack (1941) and others. Male crayfish are classified as Form I, II or "juvenile" on the basis of the morphology and function of the gonopods. Mature males with sexually-functioning gonopods are designated as Form I, and those with non-functioning gonopods, as Form II; immature males with non-functioning gonopods are classified as "juvenile." Most mature males change from Form I to Form II at the spring molt and revert to Form I during the summer molt in time to function during the fall mating season, remaining in Form I until the following spring. No satisfactory explanation of these changes in sexual form has been noted in the literature.

It is possible that sex hormones are involved in the delay of the spring molt of egg-carrying females and in the changes of sexual form of the male gonopods at the time of molting. However, there is no conclusive evidence for the presence of sex hormones in the crustaceans. As pointed out by Brown (1944) most of the proof for the presence of sex hormones is based on indirect results such as parasitic castration, radiation and regeneration experiments.

Materials and Methods

Most of the laboratory experiments and some field observations were made on the crayfish, *Cambarus immunis* Hagen; but a few observations were made on *C. propinquus* Girard. The stock animals were kept in lead-lined tanks supplied with running tap water and were offered chopped earthworm or liver as food. The experimental crayfish were placed in individual finger bowls which were frequently refilled with fresh tap water and maintained at room temperature.

The eyestalks were removed by excising through the basal membrane with a sharp, pointed scalpel and coagulating the open wound with an electric cautery to control hemorrhage. Evidence of an approaching molt was obtained by sacrificing an animal and examining the anterior wall of the stomach for the presence of gastroliths. The pair of gastroliths were dried in an oven at 100° C. for 24 hours and weighed to determine gastrolith size. The carapace lengths were measured from the posterior margin of the cephalothorax to the tip of the rostrum.

The technique of inducing gastrolith formation and molting by removal of both eyestalks, developed by Brown and Cunningham (1939), Kyer (1942), Scudamore (1942a, 1947) and others, permitted (1) a study of secondary sex changes in male crayfish during winter molts induced by eyestalk removal as well as during normal spring and summer molts and (2) an investigation of the role of the eyestalks (sinus glands) upon molting in egg-carrying female crayfish.

Field Observations

Collections of *C. propinquus*,[2] made from a single locality during the spring and summer, illustrate the phenomena of the delay in the spring molt of egg-bearing females and the changes in sexual form of males (Table 1). On March 27th an ice jam had flooded a stream flat and, when the water receded, great numbers of

[2] From the unpublished records of collections made by Dr. C. L. Turner in 1921 from Turtle Creek, Rock County, Wisconsin.

crayfishes remained on the flat, either dead or in a dormant condition. All the males were Form I and had hard, calcareous exoskeletons. None of the females were bearing eggs. In this particular collection there were more females than males. According to Van Deventer (1937) males slightly exceed the females in number and, during the egg-bearing period, greatly out-number the females in the active population.

Table 1

Summary of molting and changes in the sexual cycles of the crayfish, C. propinquus, as observed in random field collections

Date	Total collected	Form I males		Form II males		Females		
		Number	Condition of exoskeleton	Number	Condition of exoskeleton	Number	Condition of exoskeleton	Number bearing eggs
March 27	270	109	Hard	0	—	161	Hard	0
April 15	76	68	Hard	0	—	8	Hard	7
April 24	156	124	Hard	0	—	32	Hard	26
May 1	48	46	Hard	0	—	2	Hard	2
May 13*	46	6	Hard	28	Soft	{ 8 4	Hard Soft	8 0
June 3**	136	3	Hard	88	Soft Medium Hard	{ 26 19	Hard Soft	0 0
July 6***	69	6	Soft	26	Hard	{ 30 34	Soft Hard	0 0
Late July*** and August	Many	Many	Mostly hard	Very few	Hard	Many	Hard	None

* Spring molt of males and non-reproducing females.
** Spring molt of egg-bearing females.
*** Summer molt, both sexes.

On April 15th only eight females were secured in a collection of 76 crayfish which was made with a dip net without searching under stones. Seven of the females were bearing eggs upon their swimmerets. All the males were still Form I. Collections of April 24th and May 1st consisted chiefly of hard-shelled Form I males. The majority of the females were carrying eggs and were found hidden under stones. Apparently they had not moved from their hiding places or eaten for several days because an examination of their alimentary canals revealed no food. The few females not bearing eggs were moving around actively like the males.

By May 13th there were many cast-off exoskeletons lying in the margins of the shallow waters and practically all of these had come from Form I males but a few had come from non-reproducing females. Most of the males collected were soft-shelled and Form II, showing evidence of a recent molt. The four active females without eggs had molted recently. However, the eight females bearing eggs or young were concealed under rocks and had not molted.

Most of the males collected on June 3rd were Form II, indicating that they had completed the spring molt. The young crayfish, which up to this time had been clinging to the pleopods of the females, were in an advanced stage and many had left the females altogether. Many of the females were soft-shelled as a result of a recent molt following escape of their young, and nearly all of the exuvia lying in the shallow water were those of females that had carried eggs.

By July 6th most of the egg-bearing females had molted and some of the males had completed their second or summer molt. In late July and early August most of the males collected were Form I, indicating that the summer molt was completed. Some of the females apparently had undergone a second molt at this time and become sexually functional. In contrast to this observation, Van Deventer (1937) reported that adult females, which have borne eggs during the spring, undergo only a single molt.

Although it is difficult to delineate accurately the various events because of the length of time between collections, certain generalizations may be made regarding the life cycle of *C. propinquus* in southern Wisconsin. (1) Reproducing females deposit their eggs in early April and carry their eggs until the middle of May. The eggs hatch about mid-May and the young remain attached to the female for several days. (2) The spring-molting period of males and non-reproducing females begins after May 1st with many animals molting by May 13th and the remainder before June 3rd. (3) The spring molt of egg-carrying females, which is delayed until after the young have left the female, occurs during the month of June or about three weeks after the male spring molt. (4) The second or summer molt of most mature males and at least some females takes place during July and early August. (5) Most mature males change from Form I to Form II during the spring molt and from Form II to Form I during the summer molt.

Molting in Egg-Carrying Females

In order to determine the role of attachment of the eggs to the swimmerets in delaying molt, a number of egg-bearing female crayfish, *C. propinquus,* were placed in a large aquarium, closely simulating the natural environment, during the spring-molting period of males and non-reproducing females. The eggs were then removed from one group of females. Both those with eggs attached and those with eggs removed were given access to an abundant food supply. The egg-carrying females remained in their hiding places beneath stones in the aquarium, waving the mass of eggs attached to their swimmerets but not feeding. On the other hand, the crayfish, from which the eggs had been removed, soon began to move about freely, fed actively and molted within one or two weeks.

In experiments performed during the winter of 1941–42, twenty normal egg-carrying female crayfish, *C. immunis,* were placed in individual finger bowls and all of the eggs removed from the pleopods of ten of them; both eyestalks were extirpated from another group of twenty egg-bearing females, and the eggs removed from ten of these animals. The crayfish from each of the four groups were sacrificed (or died) and examined for the presence of gastroliths at various intervals of time. One eyestalkless crayfish in each group died of operative injury before sufficient time had elapsed for gastroliths to form (Scudamore, 1947) and so were not included in tabulating the results.

Only three of the normal egg-carrying crayfish were found to contain very small gastroliths and none molted (Table 2), even though these animals were observed for a period nearly three times longer than the eyestalkless crayfish (Table 3). The experimental period was long enough to permit gastrolith formation and molting when compared to normal pre-molt periods (Scudamore, 1947). Minute gastroliths have been observed in other normal crayfish during the winter, but their exact significance is not known. There was no essential difference in the results whether the eggs were removed or not. This experiment demonstrated that the mere removal of the eggs from the normal crayfish does not induce molting in a non-molting season.

Table 2

Influence of removing the eggs from the pleopods of normal egg-carrying female crayfish, C. immunis, upon gastrolith formation and molting from December to February

	Carrying eggs	Eggs removed
Number of animals	10	10
Average duration of experiment (days)	34.5	39.8
Number with gastroliths	2	1
Average weight of both gastroliths (mgm.)	0.25	0.06
Number molted	0	0
Average carapace length (mm.)	30.6	28.5

Table 3

Effect of bilateral eyestalk extirpation upon gastrolith formation and molting of egg-carrying female crayfish, C. immunis, from December to February

	Carrying eggs	Eggs removed
Number of animals	9	9
Average duration of experiment (days)	12.7	15.2
Number with gastroliths	9	9
Average weight of both gastroliths (mgm.)	44.80	54.19
Number molted	1	2
Average carapace length (mm.)	28.6	27.8

All of the eyestalkless crayfish had formed large gastroliths in 5–19 days after eyestalk removal and three of them had molted between 15 and 17 days after operation, even though winter is normally not a molting season (Table 3). The removal of the eyestalks resulted in gastrolith formation or molting whether the eggs were present or not. Although there was considerable individual variation, there was no significant difference in the rate of gastrolith formation in the two groups of eyestalkless crayfish (Table 4). The inhibition of molt seemed to be dependent upon both the presence of the eyestalks (sinus glands) and the attachment of the eggs to the pleopods of the female and not simply the attachment of the embryos to the female as concluded by Hess (1941).

Although the evidence suggests that the sinus gland molt-inhibiting hormone is responsible for the delay in the spring molt of egg-bearing female crayfish, there are a number of possible factors that may operate in maintaining the sinus gland activity until after the eggs hatch and the young leave the female, namely: hormonal, metabolic or nervous factors.

A female sex hormone elaborated in the ovaries or other tissues may cooperate with the sinus gland activity in the delay of molt. However, the lack of histological or experimental evidence for such glandular tissue (Brown, 1944) weakens this hypothesis. In this connection Turner (1935) reached the conclusion, on the basis of morphological studies, that the complete development of the annulus ventralis, a female secondary sex character, depends upon some ovarian tissue and the total absence of any testicular tissue. Yonge (1937) reported a cycle of histological changes in the oviducal epithelium and of secretion from the "cement" glands of the pleopods associated with egg-laying and attachment of the eggs to the pleopods of the lobster and suggested that, in the absence of nervous connections to the epithelium or the glands, the cycle of changes seemed to be controlled by hormones.

TABLE 4

Rate of gastrolith formation in eyestalkless egg-carrying female crayfish, C. immunis, during the winter

Day after eyestalk removal	Carrying eggs		Eggs removed	
	Length of carapace (mm.)	Weight of gastroliths (mgm.)	Length of carapace (mm.)	Weight of gastroliths (mgm.)
13	28.7	19.6	28.7	10.3
13	28.0	67.9	28.3	81.0
13	——	——	27.5	21.1
15	29.8	22.7	26.8	49.8
15	29.8	73.8	29.5	97.2*
16	23.2	40.2*	28.8	84.2
16	——	——	29.6	33.8
17	——	——	26.4	50.1*
19	31.5	69.7	25.0	60.2

* Indicates that animal molted.

The annual cycle of metabolic changes, which normally may initiate molting directly or through inhibition of the sinus glands, may be delayed in the egg-carrying females. The importance of metabolic factors is emphasized by the observation that egg-bearing female crayfish are largely inactive, hiding under stones and not feeding freely until after the young leave the pleopods. Furthermore, the egg-bearing females become active, feed and molt within a short period of time after the eggs are removed artificially during the male spring-molting period.

Finally, prolongation of the molt-inhibiting action of the sinus glands by impulses over nerve-reflex pathways produced by the presence of the eggs on the pleopods may explain the delay in molt. Welsh (1941) has demonstrated morphologically an innervation of the sinus glands of the crayfish from the "brain." Moreover, the tracts followed within the central nervous system of the crayfish by sensory impulses from stimulation of proprioceptors and sensory hairs of the abdominal pleopods were traced functionally by Prosser (1935), confirming the neurone paths first described histologically by Retzius (1890). These observations suggest the nervous pathways which may be involved in the reflex stimulation of the sinus glands. However, the fact that removal of only the eggs and not the eyestalks did not initiate gastrolith formation in the winter even in the warmth of the laboratory, suggests that some factor or factors other than possible pleopod-sinus-gland reflexes are involved.

Some combination of hormonal, metabolic and nervous factors seems like the most plausible explanation of this phenomenon. It is apparent that further experimentation is needed to establish the exact mechanism involved in the delay of the spring molt of egg-carrying females.

Molting and the Sexual Cycle of Males

The cycle of changes in sexual form of the first pair of abdominal appendages is illustrated by observations on molting in a single male crayfish, *C. immunis*, between April and September. This crayfish changed from Form I to Form II at the first or spring molt on May 15th and changed from Form II to Form I at the second or summer molt on July 15th.

In order to study the changes in sexual form of crayfish during the fall and winter, observations were made of changes in sexual form following molting of mature crayfish, *C. immunis,* induced by bilateral eyestalk extirpation and these changes were compared with those occurring at normal spring and summer molts. As shown in Table 5, all the crayfish became Form II after the artificially induced winter molts whether they were Form I or II before molt. The normal crayfishes changed from Form I to Form II at the spring molt and from Form II to Form I at the summer molt.. Enough Form II males for this winter experiment were obtained by selection from a large number of animals, because most of the males were Form I.

Table 5

Changes in sexual form of mature male crayfish, C. immunis, at the time of molt

Period	No. of animals	Original sexual form	Form after molt
Spring molt	12	I	II
Summer molt	12	II	I
Winter molt, induced by eyestalk removal	12	I	II
(November to March)	10	II	II

While studying spermatogenesis of the crayfish, Fasten (1914) found a seasonal variation in the size of the testes and in germ cell proliferation. The testes commenced active proliferation and increased in size in June, reached greatest activity and size in July, remained large in August with their tubules filled with spermatozoa, decreased in size in September, and remained small until the following summer. This seasonal cycle of changes in the testes of mature males is illustrated in Figure 1 together with the duration of the spring- and summer-molting periods, the seasons during which Form I and Form II mature male crayfish predominate, and the time at which copulation occurs.

The period of greatest testis activity (July–August) coincides exactly with the summer-molting period when males change from Form II to Form I. Copulation ensues a few weeks later at a time when the males have Form I gonopods and the testis tubules are filled with spermatozoa. During spring molts and during winter molts induced by eyestalk removal, when the testis size and spermatogenic activity are at a minimum, the male crayfish changes to Form II.

These observations offer circumstantial evidence in support of an hypothesis that variations in the amount of a male sex hormone, produced in the testes or other body tissues, are responsible for the changes in sexual form of the male gonopods at the time of molt. The cyclical release of such a hormone could be influenced by other internal or by environmental factors. The greatest weakness of this hypothesis is the lack of conclusive histological or experimental evidence for the presence of secretory cells within the testes. On the basis of morphological studies of the crayfish, Turner (1935) has considered that the development of aberrant secondary sex characters is largely dependent upon genetic rather than hormonal factors. However, the seasonal changes from Form I to Form II and the reverse obviously are not controlled genetically, since they occur in a single individual.

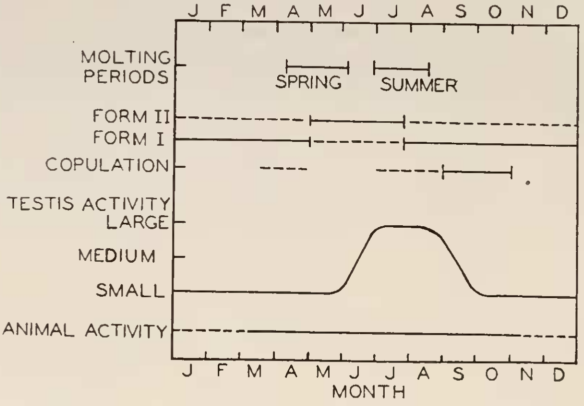

Figure 1. Diagram of certain phases in the life cycle of the mature male crayfish, *C. immunis*, demonstrating the relationship of molting periods, duration of and time of change to each male sexual form, period of copulation, periods of animal activity, and cycle of changes in testis size and spermatogenic activity. The curve of testis activity is based on the results of Fasten (1914). The solid lines (——) represent periods of predominant occurrence; broken lines (– – –), periods of occasional occurrence.

Proof for the existence of male, as well as of female, sex hormones must await histological evidence of secretory cells in the gonads or other tissues and the establishment of definite endocrine functions of these gland cells by surgical extirpation, implantation and injection of specific gland substances. However, observation and experimentation investigating the seasonal changes in the male gonopods represents a promising method for studying the problem of the existence of male hormones in crustaceans—a problem which is far from satisfactorily settled at this time.

Summary

1. The phenomena of the delay in spring molt of egg-carrying females and of the changes in sexual form of males at the time of molt are described and illustrated by field observations on the crayfish, *C. propinquus*.

2. Removal of the eggs from the pleopods of egg-bearing females, *C. propinquus*, during the male spring-molting period results in an earlier onset of molting.

3. The delay in the spring molt of egg-carrying female crayfish, *C. immunis* and *C. propinquus*, is regulated by the action of the molt-inhibiting hormone of the sinus glands.

4. Various factors, that may operate to maintain the sinus gland activity until after the eggs hatch and the young leave the female, are discussed.

5. The changes in sexual form of male crayfish, *C. immunis,* at the time of spring and summer molts, and during winter molts induced by eyestalk extirpation are described.

6. Evidence is presented supporting an hypothesis that a male sex hormone, elaborated in the testes or other tissues, may regulate the cycle of changes in sexual form at the time of molt.

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